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Global Expression Studies of Yersinia Pestis Pathogenicity

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CBNP FY02 Annual Report

Global Expression Studies of *Yersinia pestis* pathogenicity

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Objectives.

The aim of these studies continues to be the investigation into the molecular mechanisms that underlie the virulence process in *Yersinia pestis*. In particular, the focus of this work centers on the identification of novel genes and pathways responsible for the pathogenic properties of this organism. In spite of more than four decades of intense investigation in this field, the dilemma as to what makes *Y. pestis* such a virulent and lethal pathogen remains unanswered. The method being employed makes use microarray technology (DNA chip) that enables the examination of the global activities of the whole complement of genes in this pathogen. Two primary resources available to the investigators (one directly obtained from a separate CBNP-funded project) make these studies possible: 1) Whole genome comparisons of the genes in *Y. pestis* and its near neighbors with attenuated or non pathogenic characteristics, and 2) the ability to duplicate *in vitro*, conditions that mimic the infection process of this pathogen. This year we have extended our studies from the original work of characterizing the global transcriptional regulation in *Y. pestis* triggered during temperature transition from 26¡C to 37¡C (roughly conditions found in the flea vector and the mammalian host, respectively) to studies of regulation encountered during shift between growth from conditions of neutral pH to acidic pH (the latter conditions, those mimic the environment found inside macrophages, a likely environment found by these cells during infection.).

For this work, DNA arrays containing some 5,000 genes (the entire genome of *Y. pestis* plus those genes found uniquely in the enteropathogen, and near neighbor, *Y. pseudotuberculosis*) are used to monitor the simultaneous expression levels of each gene of known and unknown function in *Y. pestis*. Those genes that are up-regulate under the experimental conditions represent genes potentially involved in the pathogenic process. The ultimate role in pathogenicity of those candidate genes uncovered from these studies will be further ascertained by direct knock outs (gene inactivation) and by *in vivo* studies using an animal model.

Discovery of new virulence factors in *Y. pestis* will directly impact the development of new signatures for detection and geo-location since it will help us to understand and identify those genes that essential in making the organism pathogenic. These are genes that cannot be altered or removed from the pathogen and as such constitute the best type of signature that we can utilize in their detection and identification. Applications such as this will also enable the utilization of similar technologies to study other pathogens such as Francisella and Brucella, for which we know substantially less in terms of their modality of virulence.

FY02 progress:

In the past year, reliable, high-yield method for mRNA isolation and purification that generates sufficient amounts of RNA free of DNA and protein contaminants have been developed. Appropriate buffered media that would enable transition experiments at

varying pH starting at neutrality and extending to pH 4.0 were designed and optimized. These experiments determined that the lowest pH at which *Y. pestis* still shows substantial growth is pH 5.5 (Fig.1). Growth experiments during pH 7 to 5.5 shift were conducted for both *Y. pestis* and *Y. psudotuberculosis* under the identical conditions and appropriate hybridizations against the 5,000-gene chip were performed with RNA obtained at 1 and 4 hrs after the shift.

These preliminary experiments have lead the discovery of some 37 genes that are up-regulated at pH 5.5 (at least 2 fold with a 2-46-fold range) and some 38 genes that are up-regulated at pH 4.0 (see Table 1 for selected pH regulated genes). Noteworthy among the genes up-regulated at pH 5.5 are: the pH 6 antigen precursor, an adhesin that has been previously known to be up-regulated at acidic pH, and the three subunits encoding the enzyme urease. Interest ling, the urease is often no-functional in *Y. pestis* on account of a defect in one of the genes required for urease expression. It was furthermore discovered that both the catalase gene (*KatA*) and the catalase-peroxidase gene (*katY*) two enzyme presumably contributing to detoxification of free radicals and damaging oxygen species produced in macrophages were both up-regulated. This finding supports the likely involvement of these enzymes in the virulence process. At least five genes encoding proteins with no known function were determined to be up-regulated at pH 5.5. These are potential good candidates for genes having a role in pathogenicity in *Y. pestis* and will be further examined by knockouts and animal studies (as a result of these, and related CBNP and internally funded studies of this nature, an NIH grant has been awarded to V. Motin to conduct these type of studies). Those genes up-regulated at pH 5.5 are an entirely different set of genes than those up-regulated at pH 4.0. At least 14 genes found only in *Y. pseudotuberculosis* were upregulated at this latter pH and only 4 genes of this organism were up-regulated at pH 5.5.

A subset of genes that is both regulated during pH shift as well as during temperature shift has been identified. This set might represent the best new candidate virulence genes found by these studies.

In parallel with the bacterial response during conditions mimicking infection, and with the support of internal laboratory funding (LDRD), we have begun work to study the host response to Yersinia infection. For this work human dendritic cells in culture have been subject to infection by several Yersinia species. Using commercially available DNA chips (Affymetrix) containing 1000s of human genes we have determined the transcriptional response mounted by the human cells in culture during Yersinia infection. A pattern of genes that respond at 1, 2, 4 and 10 hrs after *Y. pestis* infection are shown in Figure 1). We expect that a differential pattern of response to a given pathogen, might allow in the future the development of an early warning system for exposure to BW agents.

Future Outlook: Next year we will continue validating the results obtained during temperature and pH shift experiments. Some of these putative genes uncovered may be

tested by direct knockouts. However, much work in the area of temperature and pH response remains to be done. We will focus our efforts at studying the effect on the expression patterns of known *Y. pestis* mutants affected in virulence. Such studies will serve to potentially elucidate the steps in the virulence pathway affected by these mutations. We will direct the best part of our effort to study host response using knockouts: the effects of various virulence factors on cellular response will be analyzed using knockout strains of *Y. pestis* in dendritic cells. We will compare a strain lacking the V antigen which causes an anti-inflammatory response (Motin et al 1995) to wild type as well as strain lacking other activities encoded by unique genes of *Y. pestis* (these have been identified by our *Y. pseudotuberculosis- Y. pestis* comparative sequencing (manuscript in preparation). These types of experiments should shed important light into the virulence pathway of *Y. pestis.*

Finally, we will undertake comparative host response to various pathogens: We plan to study the response of two strains related to *Y. pestis*; *Y. enterocolitica*, and *Y. pseudotuberculosis.* Although genetically very similar to *Y. pestis*, they do not cause plague. We will compare the expression profiles of these related strains in dendritic and other appropriate cells to understand how the cellular response to these pathogens differs. Pathogen with a different virulence mechanism (*Brucella abortus, Francisella tularensis* and hantavirus) will be used to extend the spectrum of response being examined.

Project report sidebars

By unraveling the pathways that contribute to virulence, it will be possible to elucidate the reasons why this plague-causing bacterium is so virulent and lethal.

Understanding what human genes get activated first during bacterial infection will help the development of methods for early warning and detection.

Citations

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2. V.L. Motin, A.M. Georgescu, J.P. Fitch, J.B. Garnham, B.A. Sokhansanj, L.L.Ott, M.A. Coleman, B.J. Marsh, J.M. Elliott, L.M. Kegelmeyer, A.J. Wyrobeck, T.R. Slezak, R.R. Brubaker, and E. Garcia. Genome-Wide Expression Profiling of *Yersinia pestis* During Low-Calcium Response. Abstracts, p. 159.The 102th General Meeting of the American Society for Microbiology, Salt°°Lake City, UT, May 19-23. 2002. (UCRL-JC-146479-ABS)

3. Motin, V.L. Georgescu, A., Elliott, J. Hu, P., Worsham, P.L. Song, J., Brubaker, R.R. and E. Garcia. 2002. Genetic Variability of *Yersinia pestis* Isolates as Predicted by PCR-based IS100 Genotyping and Analysis of the Structural Gene Encoding Glycerol-3-Phosphate Dehydrogenase (*glpD*)., J. Bacteriol.184:1019-1027

4. Iwobi, A., Rakin, A., Garcia, E., Heesemann, J. 2002. Representational difference analysis uncovers a novel IS*10*-like insertion element unique to pathogenic strains of *Yersinia enterocolitica.* FEMS Microbiol. Letters 210:251-255.

Figure 1 (Garcia, E.)

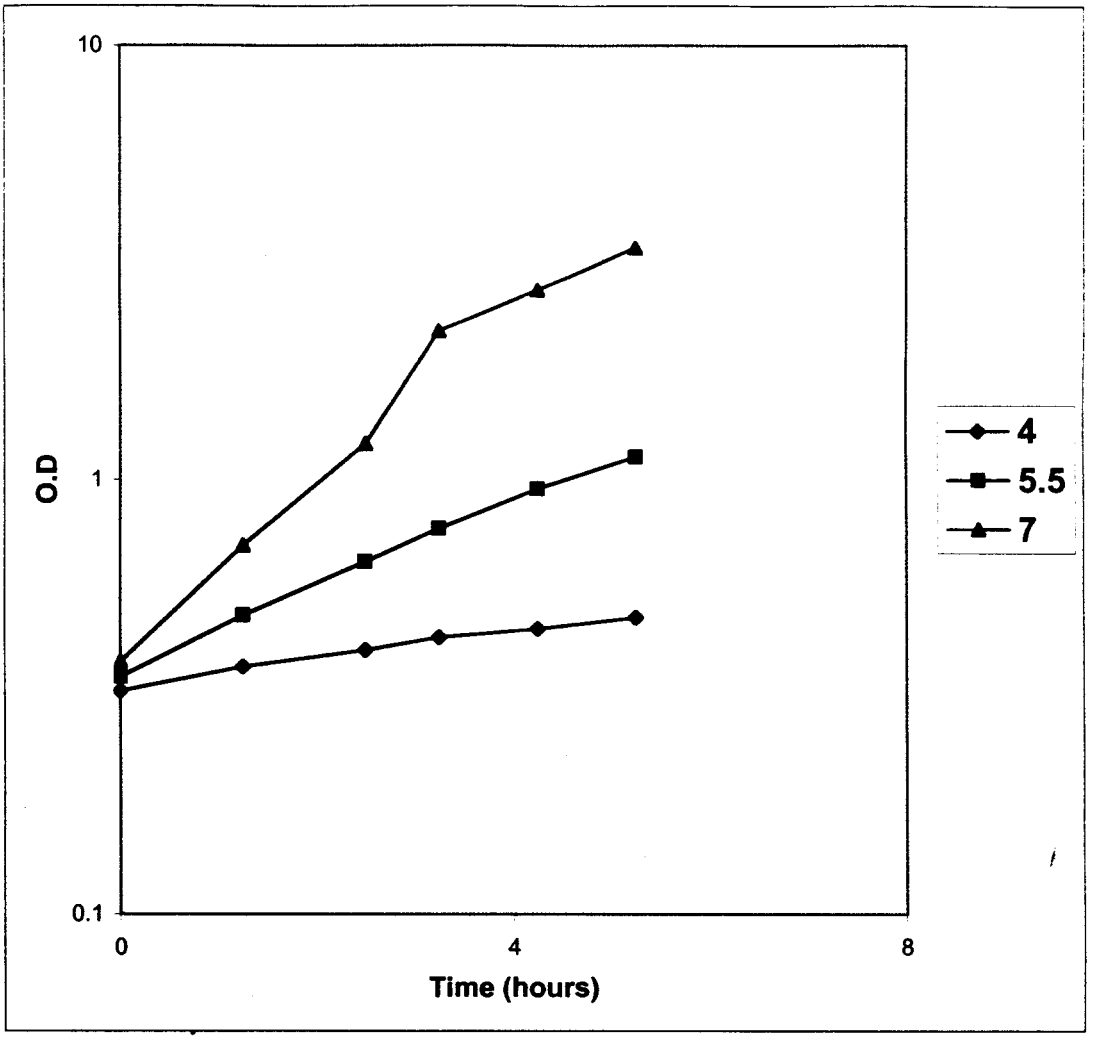
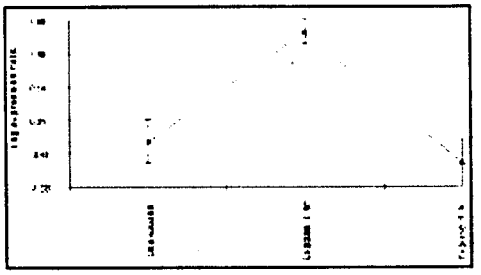
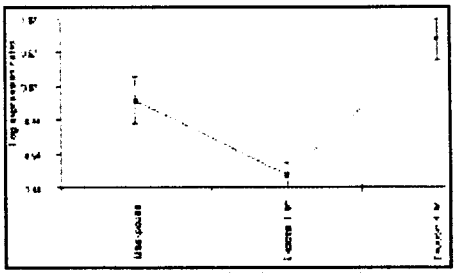


Figure 1. Growth Curve of *Y. pestis* cells grown at different pH

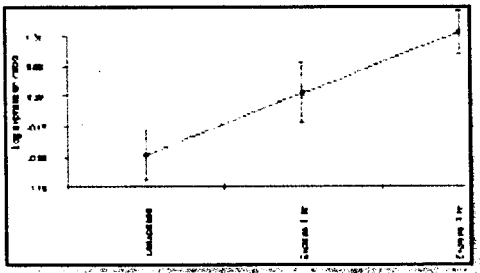
Group1. 183 genes up-regulated after 1 hr exposure, returned to normal @ 4 hrs.



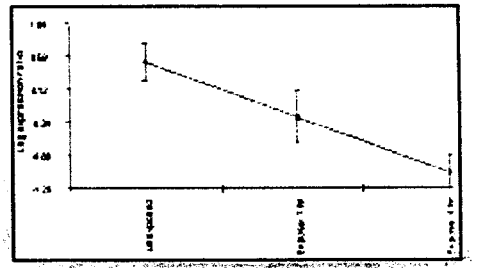
Group2. 175 down-regulated after 1 hr exposure, returned to normal @ 4 hrs.



Group3. 229 genes up-regulated after 1 hr exposure, further elevated @ 4 hrs.



Group4. 140 down-regulated after 1 hr exposure, further repressed @ 4 hrs.



Genes in *Y. pestis* induced at pH 5.5 (>2 fold)

Gene name	Product/Function	Fold (ratio)
YPO1303	**pH 6 antigen precursor (antigen 4) (adhesin)**	46.14
YPO2667	urease alpha subunit	7.33
YPO2665	urease gamma subunit	6.36
YPO1304	chaperone protein PsaB precursor	5.97
YPO2666	urease beta subunit	4.24
YPO3098	probable glycosyltransferase	3.63
YPO2668	urease accessory protein	3.54
YPO0833	putative phospho-sugar isomerase	3.35
YPO2672	putative urea transporter	3.23
YPO3716	maltose transport system permease protein MalG	3.13
YPO3987	putative exported protein	2.82
YPO2221	**aconitate hydratase 1**	2.59
YPO1994	**hypothetical protein**	2.56
YPO2671	urease accessory protein (pseudogene)	2.52
YPO1996	**hypothetical protein**	2.48
YPO1995	**hypothetical protein**	2.48
YPO3950	putative membrane protein	2.38
YPO1783	Ferritin	2.25
RpoH	Heat shock sigma factor	2.22
YPO2680	PTS system, cellobiose-specific IIA component	2.21
YPO2540	putative thermosensitive gluconokinase	2.20
YPO1207	**Catalase (katA)**	2.14
YPO2678	PTS system, cellobiose-specific IIB component	2.12
YPO3319	**catalase-peroxidase**	2.09
YPO3370	**cysH-phosphoadenosine phosphosulfate reductase**	2.05
YPO2754	conserved hypothetical protein	2.03
YPO1831	flagellar hook-basal body complex protein FliE	2.03

Genes induced at pH 4.0 (>2 fold)

Gene name	Product/Function	Fold (ratio)
YPO2241	putative exported protein	5.21
YPO2027	conserved hypothetical protein (pseudogene)	2.14
YPO3474	hypothetical protein	2.10
Y1042_orf81	NA	2.10
YPO0238	mechanosensitive ion channel	2.09
YPO1383	formate acetyltransferase 1	2.06
YenI	NA	2.04
YPO2142	Na+/H+ antiporter	2.04

***Genes in bold may be involved in virulence and/or were induced also at 37¡C**